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Research report

Development of violence in mice through repeated victory along with changes in prefrontal cortex neurochemistry

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Abstract

Recent reviews on the validity of rodent aggression models for human violence have addressed the dimension of pathological, maladaptive, violent forms of aggression in male rodent aggressive behaviour. Among the neurobiological mechanisms proposed for the regulation of aggressive behaviour in its normal and pathological forms, serotonin plays a major role. However, the results on the detailed mechanism are still confusing and controversial, mainly because of difficulties in extrapolating from rodent to human psychopathological behaviour. Our aim was to investigate the involvement of serotonin in pathological aggression. We subjected mice genetically selected for high (SAL, TA, NC900 lines) and low (LAL, TNA, NC100) aggression levels to a repeated resident-intruder experience (RRI mice) or to handling as a control procedure (CTR mice). Pathological aggression parameters we recorded were aggression towards females and lack of communication between the resident and its opponent. In the same mice, we measured the monoamine levels in the prefrontal cortex, a brain region strongly involved in the regulation of motivated behaviour. Our results show that SAL mice augmented their proneness to attack and showed the most pathological phenotype, with disregard of the opponent's sex, high territorial behavioural patterns, and low sensitivity to signals of subordination. In contrast, TA and NC900 augmented their proneness to attack and low discrimination of the opponent's signals, without showing offence towards females. After repeated resident-intruder experience, serotonin levels in the prefrontal cortex were significantly lower in SAL than in LAL whereas dopamine turnover was significantly higher, compared to CTR mice. Serotonin turnover was significantly reduced in all RRI mice, with no strain differences. Noradrenaline was significantly lower in aggressive mice of the TA and NC900 lines compared to their low-aggressive counterparts, with no effect of the repeated resident-intruder experience. We conclude that social experience changes prefrontal cortex neurochemistry and elicits pathologically aggressive phenotypes.

Keywords: Violence; Mice; Prefrontal cortex; Serotonin; Dopamine; Resident-intruder; Male-to-female aggression; Lag-sequential analysis

1. Introduction

Despite a large number of studies, the neurobiological determinants for the development of pathological aggression and violence are still far from clear. Usually in the context of resource competition, ritualized forms of aggressive behaviour are displayed that are under tight inhibitory reconciliation and appeasement mechanisms and hence do not frequently result in serious harm and injury [4,15]. However, in certain individuals under particular conditions, the motivation for aggression may escape control and escalate into violent and indiscriminate forms which inflict a considerable burden on society. Animal models for escalated aggression or violence often focus merely on the intensity factor as a parameter to delineate the escalation, for example measuring number of attacks or attack duration and frequency. Recently, it has been suggested that in order to mimic the human psychopathology, other dimensions of rodent aggressive behaviour should be measured, such as the loss of discrimination revealed by attacks towards females, attacks on vulnerable regions and/or insensitivity towards the social submission signals of the opponent [17]. In humans, the loss of discrimination is often expressed by violence against women, particularly expressed in a domestic context. Sexual abuse and domestic violence have severe physical and psychological effects on the victims and represent a major problem in our society [9,32].

In laboratory conditions, intensively aggressive mice have been obtained through bidirectional artificial selection for aggression. Using this method, several genetic lines of mice

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selected for high (SAL, TA, NC900) or low (LAL, TNA, NC100) aggressiveness were generated [43,24,8]. In mice, highly aggressive behavioural phenotype is present in a semi-natural population and is selected under certain environmental conditions because of high fitness due to better access to/defense of food and females [44]. Beyond a clear genetic predisposition to aggression, escalated aggression can also be achieved through frustration, instigation, alcohol consumption [11] or repeated social victory experiences [16,13,23]. In particular, repeated victories may reinforce the use of aggressive behaviour in order to achieve better position in social hierarchies. In such experiments, aggression increases in terms of duration and frequency, while attack latency decreases [31]. It has been suggested that these escalations mimic the development of psychopathology, although it is not known to what extent the genetic aggressive predisposition is necessary for this process and therefore a risk factor for violence. Aggression by males towards females has been observed in the SAL and TA aggressive selection lines. SAL males attacked females more than LAL and this behaviour was enhanced with a period of repeated daily winning experiences against male intruders [5]. TA male mice attacked females more than TNA when they had previously been isolated [30]. To our knowledge, aggression by males against females has never been studied in the NC lines. Other studies on psychopathological elements of inter-male aggressive behaviour portrayed SAL mice as antisocial and violent compared to non-aggressive LAL, and TA as insensitive to social cues compared to TNA [18,35,45].

From clinical and preclinical studies it is known that, among the various central neurotransmitters and neuromodulators, serotonin plays a major role in the control of aggression and more generally of motivated behaviours, whereas monoamines in general play a crucial role in mood regulation [29]. Brain serotonin is produced by neurons whose cell bodies form the raphe nuclei and whose projections reach virtually all brain areas, including the prefrontal cortex where the innervation is considerable. Among the brain areas involved in the regulation of aggression, prefrontal cortex is particularly interesting, since it is associated with aggressive psychopathologies. Reduced activity of the prefrontal cortex, in particular its medial and orbitofrontal portions, has been associated with violent/antisocial aggression [33,6]. In laboratory rats, serotonin dynamics in the prefrontal cortex was associated to the execution of aggression [41]. Male mice of the SAL and TA aggressive lines had significantly lower serotonin tissue levels in the prefrontal cortex than the low-aggressive LAL and TNA lines, while the difference was not so pronounced in the NC lines [10]. These lower serotonin levels are associated with a higher inhibitory activity of the major short- and long-looped feedback regulatory mechanism of serotonin cells, the 5-HT_{1A} receptor, as an autoreceptor in the case of SAL mice and as a postsynaptic receptor in the case of the TA mice [39,10].

The first objective of our study was to escalate aggression levels from normal to pathological in mice genetically selected for high and low aggressiveness. We subjected SAL, LAL, TA, TNA, NC900 and NC100 male mice to repeated daily resident-intruder (RI) experience and tested aggression against females as a criterion of the development of pathological aggression. To confirm the interpretation of the results in terms of the possible development of a lack of social communication skills in highly aggressive mice, the sequential structure of behaviour during the last resident-intruder interaction was analysed in detail. Our second aim was to elucidate the involvement of prefrontal cortex serotonin levels in the development of pathological aggression. We measured serotonin and monoamine levels in the prefrontal cortex of the same mice and compared them with those of control mice of the same selection lines that never experienced any male–male interaction.

2. Materials and methods

2.1. Animals

Male mice ($n = 60$) from six different lines (SAL, LAL, TA, TNA, NC900 and NC100) obtained through three independent selection breeding programs (SAL, LAL = Groningen; TA, TNA = Turku, Finland; NC900, NC100 = North Carolina) were used as experimental subjects. They were kept from weaning (3–4 weeks of age) in familiar unisexual groups in Makrolon Type II cages, and subsequently (6–8 weeks of age) housed in pairs with a familiar female to avoid social isolation. Female mice ($n = 60$) from the same lines were used as female intruders. MAS-Gro male mice were used as male intruders, since they exhibit a neutrally docile phenotype in a male–male confrontation. Male and female intruders were housed in unisexual groups of four animals. All the mice were at least 3–4 months old at the beginning of the experiment. Rodent food pellets (AMII, ABDiets, Woerden, The Netherlands) and water with a low chloride content were accessible *ad libitum* during the whole experiment. All the mice were kept under controlled 12/12 h light/dark cycles and a constant temperature of $22 \pm 2\,^{\circ}C$.

The experiment consisted of a series of behavioural tests in the following order: female attack novel cage test 1, female attack home-cage test 1, repeated resident-intruder (RRI) test, female attack home-cage test 2, and female attack novel cage test 2. All tests were performed with the approval of the Institutional Animal Care and Use Committee of the University of Groningen (DEC n. 4540A).

2.2. Repeated resident-intruder test

To obtain pathologically aggressive mice, 30 male mice underwent a repeated resident-intruder treatment (RRI group). This consisted of nine male–male resident-intruder [12,43] experiences, one each day, carried out at the same time of the day (at the beginning of the dark period) in a test cage ($75 \times 29 \times 27$), where each male had previously been housed with a female, in presence of food and water. Each day, 1 h before the RI experience, the female partner was removed from the cage. Subsequently, a naive male intruder was placed in the cage and the attack latency, i.e., the time it took the resident to attack the intruder, was scored. The intruder was removed from the experimental cage immediately after the first attack from the resident. If there was no attack, the test was stopped after 10 min and a score of 600 s was given. In the rare event of an attack from the intruder on the resident (observed in less than five cases, in the LAL and NC100 lines), the test was stopped immediately in order to avoid any defeat experience, and a score of 600 s was assigned. In the last resident-intruder experience (RI9), the intruder was left for 5 min in the resident cage and a video recording was made for subsequent behavioural analysis.

The remaining 30 experimental male mice were used as a control group (CTR). They were not subjected to the RRI paradigm, but instead were briefly handled and their female partners removed for the same duration every day as for the RRI group.

2.3. Aggression against females

As one criterion of pathological aggression, offensive behaviour by males towards females was examined before and after the RRI experience, both in a novel cage against a familiar female and in the home-cage against an unfamiliar female. The first test measures the effects of a mild stressor (a novel environment)

on the aggressive behaviour against females whereas the latter tests the situation of an unknown female in the own territory.

2.4. Female attack novel cage (FN)

At the beginning of the experimental session, each male–female pair was housed in a novel test cage provided with new bedding and nesting material, food and water. Any attack from the male mouse towards the female cage-mate within 30 min was scored. At the end of the RRI experimental session, a second test was performed when the male–female pairs were housed in standard cages.

2.5. Female attack home-cage (FH)

This test was a modified version of a previously described resident-intruder test [12,43] in which the intruder was an unfamiliar female mouse instead of a male. Two days after being housed together with a female in a test cage in which he could establish his territory, the resident male underwent a first FH test (FH1). Briefly, at the beginning of the dark phase, the familiar female was removed. One hour later, an unfamiliar female of the same line as the resident male was introduced in the cage containing the male. The interaction between the male resident and the female intruder lasted 5 min and was video-recorded for subsequent behavioural analysis. Immediately after the test, the familiar female was reintroduced after the removal of the unfamiliar one, which was returned to its home-cage.

The FH test was repeated after 10 days (FH2). In this test the same females were used as in FH1 but in a different order, so that each male would never encounter the same female intruder. In both FH1 and FH2, each female was checked during handling for oestrus or non-oestrus (dioestrus, metaoestrus or anoestrus). A small brush was gently inserted 0.5 cm into the vagina and rotated gently. The material obtained was smeared on a microscope slide, with three samples taken for each animal. A drop of methylene blue was added to each sample and allowed to dry overnight at room temperature. The next day, the samples were examined under a light microscope. Oestrus was inferred from a predominance of keratinized epithelial cells, and non-oestrus (dioestrus, prooestrus, metoestrus and anoestrus) inferred otherwise.

2.6. Behavioural analysis of RI9

To further characterise pathological aggression, the interaction between a male resident and a male intruder during the last confrontation was examined. Behavioural analysis was performed using The Observer 5.0 (Noldus Information Technology b.v.), using low speeds (5× and 20×) when required. The behavioural states of the resident and the intruder in the last male–male interaction (RI9) were scored. Since the attack latencies were different among the mice, and they represented the first part of the total 5 min of interaction, in order to analyse the patterns in the aggressive behaviour between all the animals in a consistent amount of time, we scored 1 min of interaction. The first minute after the first attack was chosen because it has the highest frequency of aggressive behaviour. The behavioural elements "attack", "chase", "threat", "social exploration", "mounting", "non-social behaviours" and "inactivity" were scored for the resident animal, while "submission", "move-away", "social exploration", "non-social behaviours" and "inactivity" were scored for the intruder. The interactions were scored with a two-subject configuration, on separate occasions for the resident and the intruder, at low speed and with time synchronization in order to precisely identify the start and end of each behaviour. For each interaction, two channels of simultaneous behaviour sequences were thereby obtained to be analysed separately for the resident and intruder. The two channels were synchronized and considered as one sequence for an event-lag based sequential analysis, in order to identify predictable interaction patterns between the resident and the intruder (see data analysis and statistics for details).

2.7. Brain-tissue preparation and HPLC

In order to avoid any acute stress effect, all the animals were sacrificed under CO_2 anaesthesia 24 h after the last behavioural test, the female attack novel cage test 2. The male residents were weighed and decapitated and their brains removed. The prefrontal cortex was dissected from each brain, frozen in liquid nitrogen and stored at −80 °C. The PFC samples were homogenised in 1 ml 0.1 M perchloric acid for 60 s and centrifuged at 14,000 rpm for 10 min at 4 °C. The supernatant was removed and stored for 1–2 days at −80 °C in order to avoid serotonin degradation, since −80 °C storage, even for a few weeks, was shown to yield comparable results (unpublished observations). One hundred microlitres of supernatant were subsequently injected into a high-performance liquid chromatography (HPLC) column (Gemini C18 110A, 150 mm × 4.60 mm, 5 u, Bester) connected to a detector (analytical cell: ESA model 5011, 0.34 V). The mobile phase consisted of 62.7 mM Na_2HPO_4, 40.0 mM citric acid, 0.27 mM EDTA, 4.94 mM HSA and 10% MeOH (pH 4.1). Known amounts of monoamines were run in parallel for standardisation. Monoamine levels were calculated as ng/g tissue.

2.8. Data analysis and statistics

Attacks towards females in the FN and in the FH tests were expressed as ratio of attacking to non-attacking animals in each line and tested for significant line differences using an exact test of independence for a 2 × 6 contingency table [22,28]. Since the SAL mice already attacked females in FH1, their attack/threat ratio was calculated and analysed using ANOVA for repeated measures, with "test" as a within-subject factor (female attack in home-cage 1 vs. 2) and "group" as a between-subjects factor (RRI vs. CTR).

For the RRI data, attack latency data were analysed using ANOVA for repeated measures with "day" (nine levels) as a within-subject factor and "type" and "selection" as between-subjects factors. The duration and frequency of the behaviours scored in the RI9 test were analysed within the aggressive and low-aggressive lines using a two-way MANOVA, with "selection" as a between-subjects factor. *Post hoc* analyses were performed using a *t* test for independent samples in the case of two samples, Tukey's test in the case of multiple comparisons and a paired *t* test in the case of repeated measurements. Furthermore, an analysis of the sequential structure in RI9 behavioural sequences was performed according to the first-order Markov chain analysis model [42]. Briefly, after grouping together all the animals of each line, matrices with first-order transition numbers across behaviours and subjects were obtained using the event-lag-sequential analysis module from The Observer (Noldus Information Technology b.v.). The matrices were subsequently tested using MatMan (MfW version 1.1: Noldus Information Technology 2003; earlier version described in [14]) for independence through calculation of adjusted residuals and the χ^2 test. Residuals were considered statistically significant when $p < 0.05$ after a sharper Bonferroni correction [19]. High positive (negative) significant residuals indicate behavioural transitions that occur more (less) frequently than expected by chance on the basis of the row and column totals. Kinetograms were constructed to show which behaviours enhance the probability of occurrence of other behaviours (high positive residuals) or inhibit the occurrence of other behaviours (high negative residuals), relative to the overall occurrence of these behaviours. Differences between the lines were analysed qualitatively through comparison of the diagrams.

The HPLC data on the monoamine amounts and their turnover in the prefrontal cortex were analysed using a two-way ANOVA within each selection using "group" (two levels: control and RRI-treated) and "type" (two levels: aggressive and low-aggressive) as between-subjects factors.

3. Results

3.1. Repeated resident-intruder (RRI) test

The changes in attack latency during the RRI experience are shown in Fig. 1. Aggressive and low-aggressive mice from different selection lines were affected in a significantly different manner by the daily experience, as found in the "day × selection × type" interaction effect (multivariate test, Hotelling's trace: $F_{(16,32)} = 2.22$, $p < 0.05$). Therefore separate analyses within each selection program were performed. Since the data did not meet the sphericity assumption, the degrees of freedom were corrected using Huynh-Feldt epsilon. In the Dutch

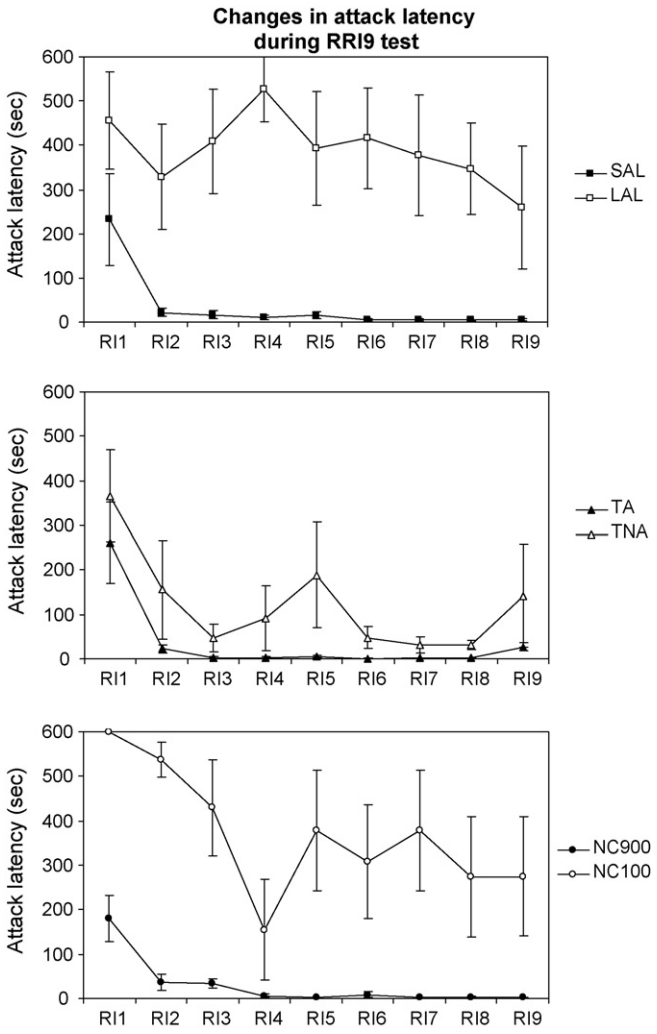

Fig. 1. Change in attack latency of aggressive (SAL, TA, NC900) and low-aggressive (LAL, TNA, NC100) mice during the repeated resident-intruder paradigm. Data are expressed as mean attack latency ± S.E.M. observed on each day of testing (from RI1 to RI9).

mice, SAL had significantly lower attack latencies than LAL throughout the whole experiment ($F_{(1,8)} = 13.48$, $p < 0.01$), as expected. The repeated resident-intruder procedure reduced the attack latency in SAL and LAL mice ($F_{(5,47)} = 2.34$, $p < 0.05$), but the overall reduction was not significantly different between the lines. In contrast, the overall attack latency of TA did not differ from that of TNA mice, but as in SAL–LAL mice it was significantly reduced during the 9-day procedure ($F_{(4,36)} = 6.97$, $p < 0.001$), with no significant difference in the overall change. In the NC lines, NC900 had lower attack latencies than NC100 ($F_{(1,8)} = 14.54$, $p < 0.01$) and a general reduction was observed throughout the 9-day experiment ($F_{(5,39)} = 5.83$, $p < 0.001$). However, the change was not significantly different between the two lines.

3.2. Aggression against females

The number of males that attacked females is expressed in Table 1 as percentages of the total number of mice of each subgroup.

Table 1
Percentage of males that attacked a female in each group and in each test performed

Line	Group	Novel cage1	Novel cage2	Home-cage1	Home-cage2
SAL	Control	80	100	80	80
	RRI	80	80	100	60
LAL	Control	0	0	0	0
	RRI	0	0	0	0
TA	Control	0	0	20	60
	RRI	0	0	0	20
TNA	Control	0	0	0	0
	RRI	0	0	0	0
NC900	Control	0	0	17	33
	RRI	0	0	0	20
NC100	Control	0	0	25	0
	RRI	0	0	0	40

3.3. Female attack novel cage (FN)

When housed in a new cage, SAL male mice exhibit offensive aggression towards their female partners within 30 min ($p < 0.001$, with SAL having the highest residual). The phenomenon is already present in the first test and remains consistent after the RRI or control experience. SAL mice also attacked oestrous females. The attack can be so violent as to cause the death of the female, as observed in one cage the day after the FN. None of the males of the other lines exhibited attack behaviour towards their females within 30 min of the novel situation.

3.4. Female attack home-cage (FH)

SAL males attacked non-familiar females both before and after the treatment. After the RRI or the control experience, some mice of the TA and NC900 lines were triggered to attack their females, suggesting a non-specificity of the treatment. Attacks from the NC100 mice were observed after the RRI period. Mice from the SAL and NC100 lines showed offensive aggression also towards oestrous females. SAL mice attacked females significantly more than mice from the other lines in the first test both in the control ($p < 0.001$, with SAL having the highest residual) and in the experienced group ($p = 0.036$, with SAL having the highest residual), whereas in the second test SAL attacked significantly more only in the control group ($p = 0.011$, with SAL having the highest residual), probably because of the increase in attacks after the experience in the other lines.

Since SAL mice fiercely attacked their females already in FH1, we analysed them separately using ANOVA. A specific effect of the RRI treatment was a significant increase in the attack/threat ratio (Fig. 2) in the SAL males that attacked females in FH1 (test × group interaction effect: $F_{(1,14)} = 10.72$, $p = 0.007$; Tukey's *post hoc* test: pre-RRI vs. post-RRI, $p < 0.05$).

3.5. Analysis of social communication in RI9

Fig. 3 shows the total duration of the behaviours of resident and intruder mice in the first minute of resident-intruder test 9.

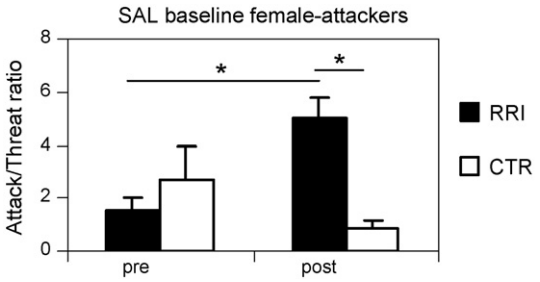

Fig. 2. Attack/threat ratio observed in SAL males that attacked females at FH1. Data are expressed as mean + S.E.M. of the RRI and CTR groups, in FH1 (pre) and FH2 (post), i.e., before and after the 9-day experience, respectively. *Post hoc* analyses: (*) $p < 0.05$.

Multivariate ANOVA found overall a highly significant "type" effect ($F_{(5,22)} = 274.7$, $p = 0.006$), a marginally non-significant "selection" effect ($F_{(5,22)} = 29.39$, $p = 0.057$) and a significant "type × selection" interaction effect ($F_{(5,22)} = 69.31$, $p = 0.012$). As expected, the aggressive mice attacked ($F_{(1,29)} = 34.97$, $p < 0.001$), threatened ($F_{(1,29)} = 8.99$, $p < 0.01$) and chased ($F_{(1,29)} = 4.6$, $p < 0.05$) the intruders more than low-aggressive mice. The mice from the low-aggressive lines spent more time in social exploration ($F_{(1,29)} = 13.75$, $p < 0.01$). Significant differences were also found in the intruders' behaviour. When exposed to aggressive mice, the intruders showed more submission and move-away behaviours ($F_{(1,29)} = 53.06$, $p < 0.001$). When exposed to low-aggressive mice, the intruders exhibited more social and non-social behaviours ($F_{(1,29)} = 4.66$, $p < 0.05$, $F_{(1,29)} = 21.61$, $p < 0.001$). Within the low-aggressive lines ("type × selection" interaction effect: $F_{(2,29)} = 3.77$, $p < 0.05$), the intruders' non-social behaviour was significantly reduced when confronted by the TNA residents, compared to LAL and NC100.

The within and between resident-intruder behavioural transitions are depicted in Fig. 4. Due to the low transition frequencies in the low-aggressive lines, the analysis was performed only in the aggressive lines. When tested for independence, the transition matrices all showed dependence across subjects and behaviours (SAL: $\chi^2(71) = 411.38$, $p < 0.001$; TA: $\chi^2(131) = 458.42$, $p < 0.001$; NC900: $\chi^2(71) = 306.02$, $p < 0.001$). The analysis of the residuals showed different within- and between-individuals dependency patterns for each line. The behaviour of SAL residents is defined by a fairly strict sequence of behaviours. SAL residents enter offensive behaviour from social exploration, reaching threat, and from threat and chase reaching attack, while occasionally they exit from threat to non-social behaviours. The communication between the resident and the intruder is well represented, with resident's threat followed by submission and resident's attack followed by intruder's move-away. The intruder's behaviour is somehow inhibiting the resident's offence, since move-away inhibits attack and submission enhances the resident's non-social behaviours.

Like SAL, TA residents show some dependence within their behaviours (chase and threat enhance attack, which in turn enhances threat, and non-social behaviours enhance social exploration), although to a much lower degree and without significant entry or exit transitions to and from the offensive behaviours. The interaction with the intruders is similar to that of SAL residents, although less striking. As in SAL, TA attack enhances the intruder's move-away, but in contrast to SAL, submission does not inhibit or enhance any of the resident's behaviours in TA. The only inhibiting transition that involves the intruder's behaviour is from move-away to attack.

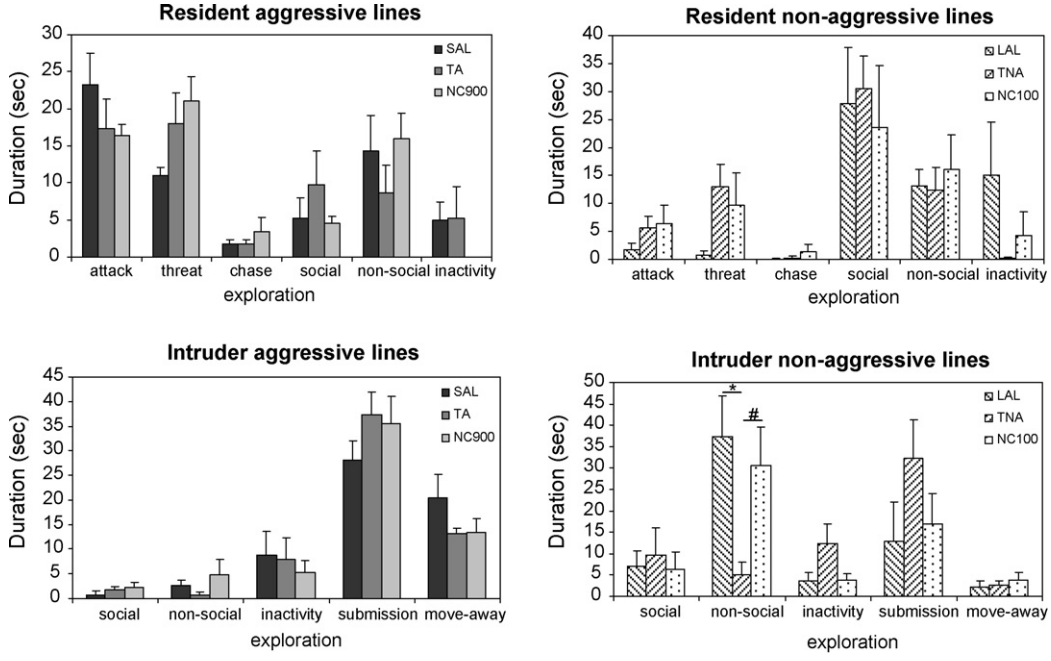

Fig. 3. Duration and frequency of the resident's and intruder's behaviour during the last resident-intruder test (RI9). Data are expressed as group mean + S.E.M. See text for statistical details and explanation. *Post hoc* analyses: (*) $p < 0.05$, (#) $0.05 < p < 0.10$.

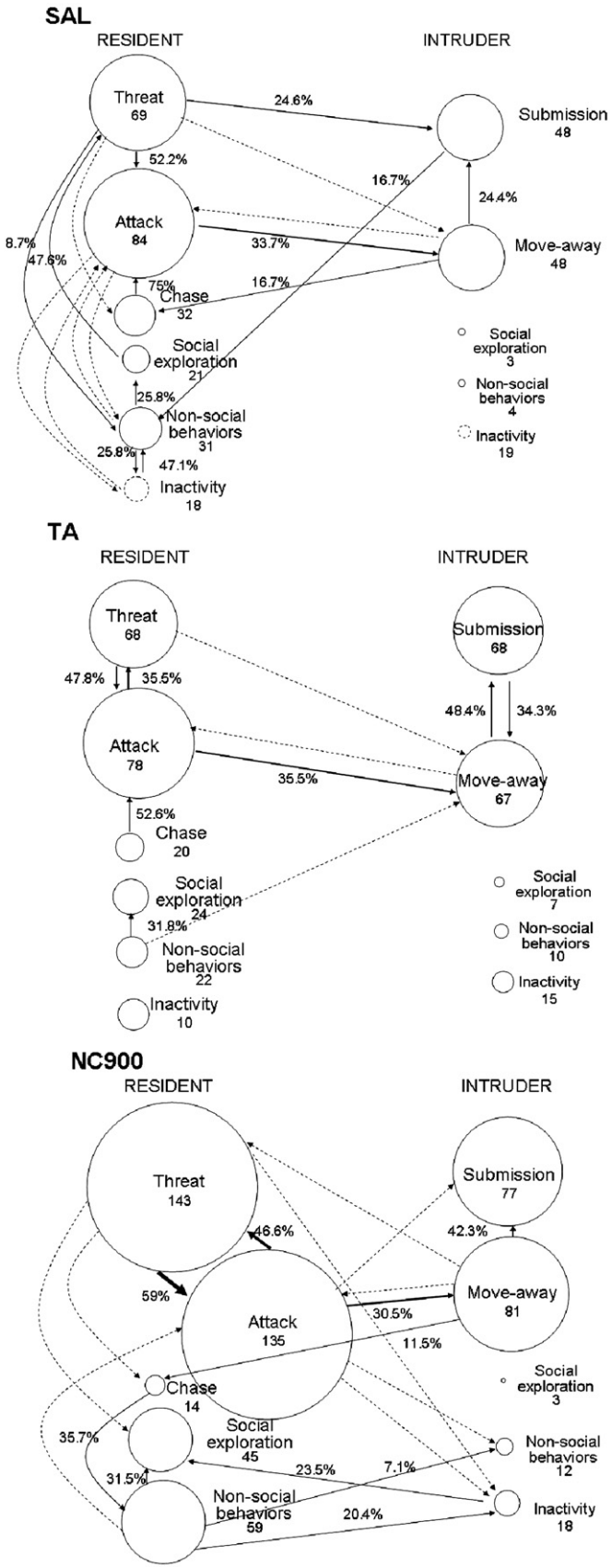

Fig. 4. Kinetogram of behavioural transitions performed in the first minute after the first attack of the last male–male resident-intruder confrontation (RI9) by male mice from the aggressive lines (SAL, TA and NC900). The areas of circles are proportional to the overall observed frequency of each behavioural event (frequencies shown). For NC900 only, the size of the boxes is reduced by factor two, since the frequencies were much higher than in the other lines. Continuous arrows indicate the transitions that occur significantly more than by chance, whereas dashed arrows indicate the transitions that occur significantly less than by chance. The widths of continuous arrows are proportional to the number of transitions. On each arrow, the observed percentage of transitions is indicated.

NC900 residents show clear within-resident dependence. The loop between threat and attack previously described in SAL and TA is also present in NC900, but in this case it is more prominent, with both behaviours also represented at high frequencies. The shift from offence to inoffensive behaviour happens as a transition from chase to non-social behaviours, while there is no evidence for an entry to offence. The interaction pattern with the intruder is more noticeable than the within-individual dependence. Similarly to SAL and TA, the resident's attack enhances the intruder's move-away, but in contrast to SAL and TA it inhibits the intruder's submission, non-social behaviours and inactivity. Differently from SAL and TA, the NC900 resident's threat inhibits inactivity. Similarly to SAL and TA, the intruder's move-away behaviour inhibits attack, but in the NC900 mice it also inhibits threat.

3.6. Biochemical data

The concentrations of noradrenaline, serotonin, dopamine and their metabolites in the PFC are reported in Fig. 5. Noradrenaline was significantly lower in the prefrontal cortex of the TA ($F_{(1,16)} = 6.89$, $p < 0.05$) and NC900 ($F_{(1,16)} = 8.42$, $p < 0.05$) aggressive lines compared to their less-aggressive counterparts. TA and TNA mice that underwent the repeated resident-intruder experience had a significantly lower amount of noradrenaline than control mice ($F = 13.88$, $p < 0.01$). No significant effects were found in the SAL–LAL model.

No significant effects were found for dopamine levels in the prefrontal cortex of any of the selection models studied. However, the dopamine turnover was affected by the RRI experience in the SAL–LAL mice in an opposite way (type × treatment effect: $F_{(1,16)} = 14.61$, $p < 0.01$), with a marginally non-significant increase in SAL-RRI mice ($p = 0.057$) and a significant decrease in LAL-RRI mice ($p = 0.027$) compared to their respective controls.

A highly significant "type × treatment" effect in the SAL–LAL serotonin content ($F_{(1,16)} = 18.75$, $p = 0.001$) is represented by a significant increase in both lines after RRI treatment ($F_{(1,16)} = 166.22$, $p < 0.001$), which was much more pronounced in the low-aggressive line. Indeed, SAL and LAL mice had similar serotonin levels in the control group, while after RRI experience SAL had significantly lower serotonin levels than LAL ($p < 0.01$). Serotonin metabolite 5-HIAA was significantly reduced in the SAL, LAL, TA and TNA mice after RRI experience (SAL–LAL: $F_{(1,16)} = 96.78$, $p < 0.001$; TA–TNA: $F_{(1,16)} = 12.77$, $p < 0.01$). Serotonin turnover was decreased in RRI mice from all the lines (SAL–LAL: $F_{(1,16)} = 96.78$, $p < 0.001$; TA–TNA: $F_{(1,16)} = 18.50$, $p = 0.001$;

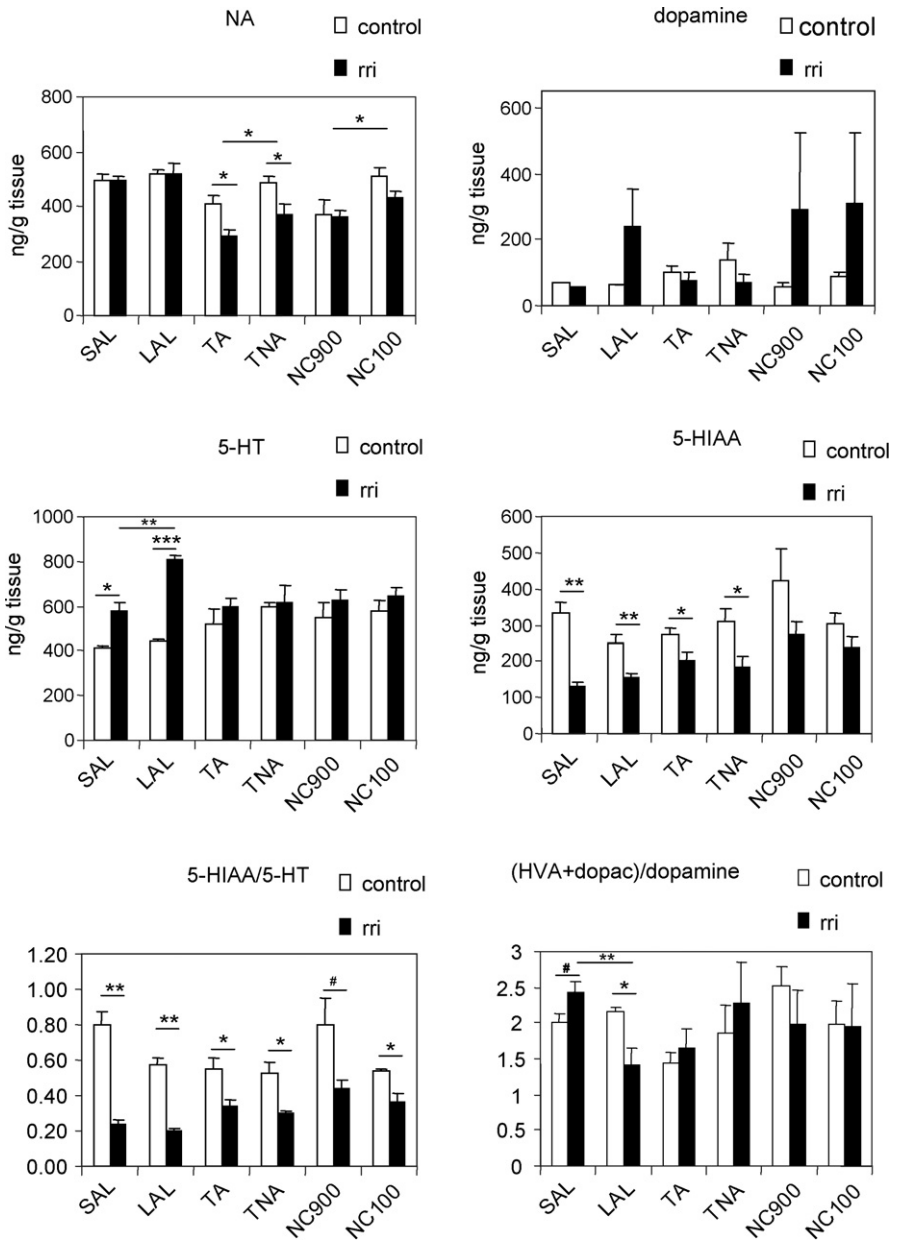

Fig. 5. Amounts of noradrenalinee (NA), dopamine, serotonin (5-HT), 5-HIAA, serotonin turnover ratio (5-HIAA/5-HT) and dopamine turnover ratio ((HVA + dopac)/dopa) in the prefrontal cortex of mice of the control and repeated-resident-intruder (RRI) groups. Data were obtained using HPLC and are expressed as mean + S.E.M. for each aggressive (SAL, TA, NC900) and low-aggressive (LAL, TNA, NC100) mouse line. (*) Significantly different at $p < 0.05$. (**) Significantly different at $p < 0.01$. (***) Significantly different at $p < 0.001$. (#) $0.05 < p < 0.10$.

NC900–NC100: $F_{(1,16)} = 6.6$, $p < 0.05$) compared to control groups, although the effect was more pronounced in SAL and LAL mice.

4. Discussion

The present study shows that differences in prefrontal cortex serotonin levels are associated with a particular type of pathological aggression, induced by subjecting wild-derived mice genetically predisposed to aggression to a repeated winning experience. This is the first study, to our knowledge, relating prefrontal neurochemical changes to a rodent's pathological aggression and genetic predisposition for aggressiveness.

4.1. Effects of social experience on aggression and violence

Repeated social experience, in the form of resident-intruder interactions, escalates aggression levels in aggressive lines, as represented by the decrease in attack latency. We could not exclude that the low-aggressive lines also escalated their aggression levels, since TNA mice reach very low attack latency values. However, while the attack latency usually correlates with the level of expressed offensive aggression, it is more an index of motivation to engage in aggressive behaviour. As a general conclusion, although all the lines were genetically selected for high versus low aggressiveness, there are differences between the three models in the way they are affected by social experience

and in the type of escalated aggression that the aggressive lines exhibit. SAL, TA and NC900 mice are all highly aggressive and their aggression levels escalate according to the "winner effect", namely the increased probability of winning an aggressive encounter following previous victories [20,31]. However, SAL mice show high levels of pathological aggression, measured as offensive behaviour towards familiar and unfamiliar females, both in the home-cage and in a novel environment, even before any male–male experience. This study is not the first to report aggressive acts from males toward females in the wild house mouse. In a wild house-mouse population observed in semi-natural conditions, natural selection favoured the evolution of a highly aggressive mouse phenotype that exhibited violence against males and females indiscriminately and against pups and juveniles as well as adults [44]. In a laboratory setting, the percentage of SAL males that attacked familiar females after 9 days of male–male resident-intruder training was significantly higher than that of LAL mice, in which this behaviour was almost absent [5]. In our experiment, most of the SAL mice attacked familiar and unfamiliar females before and after the nine male-intruder tests. In line with the previous experiment, the extremely aggressive phenotype of the SAL mice suggests a violent component comparable to that of a highly aggressive human personality. As it was described by Sluyter et al. [35], SAL mice have this and other characteristics of violent men that persistently displayed antisocial behaviour in a human longitudinal study. Aggressive animals experienced the repeated male–male resident-intruder paradigm as a repeated winning experience, since at the first attack the intruders were showing submissive postures that indicated the establishment of a dominant-subordinate hierarchy. This experience exacerbated the aggressive phenotype of SAL individuals, enhancing their attack/threat ratio against females.

It is tempting to consider the aggressive behaviour towards females as an indication of a lack of social communication skills. If this interpretation is correct, one might expect to see a lack of social communication in a male–male interaction as well. A detailed analysis of the sequential structure of the social interaction was used to study the sensitivity of the resident to the opponent's signals. The aggressive behaviour of the SAL male clearly depends on the behaviour of the opponent. However, this sensitivity is able to inhibit SAL's aggression only temporarily. SAL behaviour also showed intrinsic regulation, as seen in the high degree of intra-individual behavioural dependence, although the behavioural pattern comprises the rodent-typical sequence of behaviours shown by dominant males (from social exploration to threat and attack, or from threat to non-social behaviours and inactivity).

Different pictures are revealed by the TA and NC900 aggressive lines, obtained from mouse laboratory strains, where no attacks on familiar females were observed and very little offensive aggression was shown towards unfamiliar females. However, the latter was enhanced by daily handling and separation from the female partner (control group) and it was also seen in TNA and NC100 mice, even though they were less prone to aggressive behaviour towards males, suggesting that this effect was specific neither to repeated victory experience, nor to individuals genetically predisposed to aggressiveness. In the male–male interaction, TA mice did not show clear within- and between-individual dependence, therefore the cues for determining their behavioural shifts may not lie in intrinsic motivation or in intruder's signals. The NC900 were characterised by a higher behavioural turnover, especially in the attack/threat shifts, suggesting some uncertainty of motivation of an approach/withdrawal type. NC900 discriminate opponent's signals better than TA, although the type of communication seems to differ from that in SAL interactions. In general, both lines show less typical territorial patterns compared to SAL. To interpret this result it is important to remember that the strains from which these lines originated consisted of albino animals that had been already selected for being not so active, easy to handle, not aggressive with their cage-mates and easily bred in laboratory cages. It may be that components of the dominant phenotype were lost during this procedure. A similar trend has been well documented [13] in rats, in which escalated aggressive traits typical of a high proportion of wild rats are completely missing from the standard laboratory Wistar rat strain. TA aggression may be more sensitive to environmental cues outside the cage or highly insensitive to cues in general, whereas NC900 aggression may be largely determined by the motility/immobility of the opponent in the cage since the behavioural dependence is higher after move-away/inactivity than after submission. There is also a possibility that these two lines, having originated from albino strains, show some sensory-motor impairments compared to the dominant wild-derived mice, so their sensitivity to external cues may be disrupted by indirect factors [1,2]. In conclusion, we consider these three escalated forms of aggression as violence, because of the high intensity, persistence, and poor social communication. It seems that the three highly aggressive lines we studied may represent different types of pathological aggressive behaviours in humans, for example the aim-focused, hypoarousally driven, persistent aggression of psychopaths (represented in SAL mice) and highly emotional, reactive forms of pathological aggression (represented in TA and NC900) [6,46]. More research on the physiology and neurochemistry of these animals is needed to elucidate this concept.

4.2. Monoamine levels and types of aggression

Prefrontal cortex neurochemistry seems to vary according to the different types of aggression observed. Serotonin is the neurotransmitter mostly involved in differentiating the violent SAL type from the docile LAL, since its prefrontal cortex level is differentially changed by the male–male repeated resident-intruder paradigm in SAL and LAL mice. The fact that serotonin level was higher in the SAL–LAL animals that underwent the repeated social experience compared to the controls seems to contradict the "serotonin-deficiency" hypothesis, which states that highly aggressive/impulsive individuals show diminished serotonergic transmission activity [26,38]. However, the serotonergic system is also activated during a resident-intruder interaction, as shown previously by measuring raphe neuronal activation in rats [40] and extracellular serotonin levels by microdialysis in lizards [36,37]. Hence, the higher need for serotonin in social inter-

actions may have enhanced the baseline tissue content in SAL and LAL mice. During a social encounter, serotonin is rapidly but transiently released and, consequently, feedback mechanisms decrease the neuronal firing of the raphe nuclei. This may result in a long-term increase of serotonin synthesis by increasing the activity of tryptophan hydroxylase [7], the rate-limiting enzyme for the production of serotonin. The serotonin levels in the prefrontal cortex of the experienced SAL and LAL mice are similar to the data obtained in our previous study where SAL and LAL mice were previously tested for aggression several times throughout an experiment [10]. The lower amount of serotonin in the prefrontal cortex of SAL mice compared to LAL mice observed in that experiment might be due to the social experience the mice had during the aggression test that was performed before the experiment to screen their behavioural phenotype. The higher sensitivity of the inhibitory 5-HT_{1A} autoreceptor [10], a major feedback mechanism for the serotonergic nuclei, is a possible mechanism for the resulting lower serotonin change in the SAL mice compared to that in the LAL.

The serotonergic system was affected by the social experience in terms of a strong reduction of serotonin turnover. However, since the change was found in both the aggressive and low-aggressive lines, there is no association with victory, aggression and violence. Perhaps this change is reflected in other behavioural characteristics such as impulsivity or anxiety that we did not explore in this study.

Dopamine turnover was enhanced in SAL and lowered in LAL after the repeated social test. An earlier study showed higher nigrostriatal dopaminergic activity in SAL than in LAL mice, when all the mice were previously screened for aggressive behaviour [3]. It may be that the difference observed was due to the social experience, as shown in this experiment. An association between violent/impulsive behaviour and dopamine has previously been suggested, although the mechanism is far from clear [34]. Our data support the idea that a difference in the reduction of serotonin availability is associated with differences in dopamine neurotransmission, according to an inverse relationship [21].

In TA mice characterised by high aggression levels but little intrinsic and intruder-based regulation, aggressive behaviour is associated with low noradrenaline tissue levels in the prefrontal cortex. This result is in apparent contrast with an early study in TA and TNA mice, in which the aggressive line showed higher brainstem noradrenaline levels than the low-aggressive one [25]. The difference in brain region investigated may underlie this discrepancy. However, our results suggest a negative correlation between noradrenaline levels in the prefrontal cortex and aggressiveness, and are in line with previous findings on aggressive patients with Alzheimer's dementia [27].

In conclusion, this study shows that violence can be engendered in wild-derived mice genetically selected for aggression and that in these mice the genotype interacts with social experience, resulting in low increase in prefrontal cortex serotonin levels and dopamine neurochemistry and leading to the reinforcement of the dominant status towards a psychopathological condition. The behavioural analysis and the neurochemical data show that the genetically selected lines develop distinct violent behaviour that is associated with differential prefrontal cortex dynamics.

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